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Wilhelm, Franziska R.

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Franziska R. Wilhelm med. vet. Writing–Original draft preparation Conceptualization Investigation Methodology Da
Maria-E. Krautwald-Junghanns Prof. Dr. med. vet., Dip ECZM (Avian) ,
Victoria Ortín Piqueras DVM Advice in laboratory analysis ,
Jouni Junilla Statistical advice ,
Kerstin Cramer Dr. med. vet., Dip ECZM (Avian) Statistical advice ,
Richard A. Forsgård PhD Revision ,
Rafael Frias DVM, MSc, PhD, Assoc. Prof. (LAS) Revision ,
Thomas Spillmann Prof. Dr. med. vet., Dip ECVIM-CA Writing–Review and Editing ,
Volker Schmidt PD, Dr. med. vet., Dip ECZM (Avian & Herp) Writing – Review and Editing

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Authors:

1. Franziska R. Wilhelm, med. vet.[1] franziska.wilhelm@posteo.de

2. Maria-E. Krautwald-Junghanns, Prof. Dr. med. vet., Dip ECZM (Avian)[1] krautwald@vogelklinik.uni-leipzig.de

3. Victoria Ortín Piqueras, DVM[2] victoria.ortinpiqueras@helsinki.fi

4. Jouni Junilla[3], jouni.junilla@4pharma.com

5. Kerstin Cramer, Dr. med. vet., Dip ECZM (Avian)[1] cramer@vogelklinik.uni-leipzig.de

6. Richard A. Forsgård, PhD[4] richard.forsgard@helsinki.fi

7. Rafael Frias, DVM, MSc, PhD, Assoc. Prof. (LAS)[5] rafael.frias@ki.se

8. Thomas Spillmann, Prof. Dr. med. vet., Dip ECVIM-CA[2] * thomas.spillmann@helsinki.fi

8. Volker Schmidt, PD, Dr. med. vet., Dip ECZM (Avian & Herp)[1] * volker.schmidt@vogelklinik.uni-leipzig.de

[1] Clinic for Birds and Reptiles, Faculty of Veterinary Medicine, University of Leipzig, An den Tierkliniken 17, 04103 Leipzig, Germany.

[2] Department of Equine and Small Animal Medicine, Faculty of Veterinary Medicine, University of Helsinki, Viikintie 47, 00014 Helsinki, Finland.

[3] 4Pharma, Tykistökatu 4D, 20520 Turku, Finland

[4] Pharmacology, Faculty of Medicine, University of Helsinki, Haartmaninkatu 8, 00014 Helsinki, Finland.

[5] Education and Training Unit, Comparative Medicine, Karolinska Institute, Stockholm, Karolinska Institutet, 171 77 Stockholm, Sweden.

* These two authors contributed equally to this work.

Correspondence

Franziska R. Wilhelm, Clinic for Birds and Reptiles, Faculty of Veterinary Medicine, University of Leipzig, An den Tierkliniken 17, 04103 Leipzig, Germany. Email: franziska.wilhelm@posteo.de, Phone: +49 341 97 38416

Iohexol-based measurement of intestinal permeability in birds.

Abstract

Background

Iohexol has been successfully used as a marker to assess intestinal permeability in humans and various other mammals. The objective of this study was to evaluate the use of oral iohexol as an intestinal permeability marker in four anatomically and nutritionally diverse bird species.

Methods

Three dosages (1 ml/kg, 2 ml/kg, 4 ml/kg) of iohexol (755 mg/ml) were administered orally to each six clinically healthy pigeons and chickens at two-week intervals. Iohexol plasma

concentration was determined 45, 90 and 180 min after administration. A comparative study was performed by administering iohexol twice to each six clinically healthy cockatiels and falcons, and determining iohexol plasma concentration at 45 or 90 min after administration.

Results

The recommended iohexol dosage for permeability testing in birds was determined to be 1 ml/kg. Median plasma iohexol concentrations were 27.77 µg/ml in pigeons, 12.97 µg/ml in chickens, 14.24 µg/ml in cockatiels, and 47.81 µg/ml in falcons, 45 min after this dosage was administered. At 90 min after administration, median plasma iohexol concentrations were 40.68 µg/ml in pigeons, 21.59 µg/ml in chickens, 32.03 µg/ml in cockatiels, and 55.96 µg/ml in falcons.

Conclusions and clinical relevance

Oral iohexol was a safe and feasible marker for intestinal permeability assessment in birds. Further investigations are warranted to establish species-specific reference intervals in larger numbers of healthy birds, and to examine the use of iohexol as a permeability marker in birds with disorders associated with altered intestinal permeability.

Keywords

avian gut, gastrointestinal tract, iohexol, intestinal permeability, permeability testing

Abbreviations

^{51}Cr-EDTA	Chromium 51-labeled ethylenediamine tetra-acetic acid

ELISA	Enzyme-linked immunosorbent assay
HPLC	High-performance liquid chromatography
IBD	Inflammatory bowel disease
min	minutes

Introduction

Intestinal permeability describes an important protective function of the gut wall: it prevents potentially harmful substances entering the body from the external environment, and only allows certain molecules to pass unimpeded through the intestinal mucosa.[1] The breakdown of intestinal mucosal integrity often leads to increased permeability of the gut, which is believed to play a critical role in initiating and developing various intestinal and non-intestinal diseases.[2] Some disorders often described in the context of altered intestinal permeability include inflammatory bowel disease (IBD), celiac disease, food allergies, as well as bacterial, viral and parasitic infections.[3–7] Measuring intestinal permeability can therefore be a valuable tool to help diagnose different diseases and to reveal in part their pathophysiology. The assessment of intestinal mucosal integrity has also been used to control the progression of disease during and after therapy, to evaluate the influence of drugs and toxic substances on intestinal permeability, and to detect a relapse of disease at an early stage.[5-10]

Intestinal permeability can be measured non-invasively with an orally administered marker substance, such as lactulose and rhamnose, chromium 51-labeled ethylenediamine tetra-acetic acid (^{51}Cr-EDTA), or iohexol. After a specific time period, the concentration of the recovered marker is measured in urine or blood. Normally the marker passes through the intestinal mucosa in a very restricted manner, but in conditions when the mucosa is damaged, markers can pass at higher concentrations. Although often claimed as inert molecules, sugar probes are partially metabolized by intestinal bacteria, making precise measurements unreliable.[11, 12] ^{51}Cr-EDTA is often considered as the 'gold standard' marker for intestinal permeability measurements, but its radioactivity is a major disadvantage, limiting its use in clinical practice.[12] Iohexol is a radiographic contrast medium that has been increasingly successful in its use as an intestinal permeability marker in humans and animals.[7, 9, 13, 14] Due to its metabolic stability, non-toxicity, water-solubility, passive absorption through the intestinal

mucosa, and easy quantification, it shares many characteristics of an ideal intestinal permeability marker.[15]

In avian species, iohexol has already been used as a safe and well-tolerated contrast medium, especially for radiographic studies of the gastrointestinal tract as well as to measure the glomerular filtration rate of the kidneys.[16-18] In birds, intestinal disorders as complex as IBD are rarely described, but evidence suggests that inflammatory conditions of the intestines in the form of chronic wasting disease exist in a similar pattern in avian species as they do in mammals.[19, 20] Furthermore, feed restriction or experimentally-induced enteropathies in poultry can disrupt the intestinal barrier function, resulting in an inflammation-mediated increase in intestinal permeability.[21-23]

The purpose of this study was to evaluate the use of iohexol as a non-invasive permeability marker in healthy birds of four nutritionally diverse species, and to determine a recommended iohexol dosage for an intestinal permeability blood test after oral iohexol administration.

Materials and Methods

Animals

Twenty four clinically healthy birds were included in this study. Specifically, we used six domestic pigeons *(Columba livia* forma *domestica,* age 3.5 to 5.5 years, 610 g ± 31 g), six laying hens *(Gallus gallus* forma *domestica*, age 1.5 to 2 years, 1987 g ± 90 g, breed Lohmann brown-classic (Lohmann Tierzucht AG, Cuxhaven, Germany)), which are further referred to as chickens, six cockatiels *(Nymphicus hollandicus,* age 2 to 5 years, 105 g ± 4 g) and six falcons including four American kestrels *(Falco sparverius*, age 1.5 to 4.5 years, 100 g ± 9 g) and two Barbary falcons *(Falco pelegrinoides*, age 2 years, 531 g ± 123 g). All animals were adults, with an equal number of males and females, except for the group of chickens, which were all female. The pigeons, chickens and cockatiels were educational species owned and provided to the study by the Clinic for Birds and Reptiles of the University Leipzig, Germany. The two falcon species were privately owned by a falconer from Saxony, Germany.

Housing and Husbandry

The groups of pigeons, chickens and cockatiels were each housed in a large covered outdoor aviary with a shelter room protected from contact with wild birds and other study birds. The pigeons were fed with a seed and grain mix (Rassetaubenfutter Universal, mifuma GmbH, Mannheim, Germany), the chickens with a rough-cut grain variation (Geflügelfutter Vollkraftmehl, mifuma GmbH, Mannheim, Germany) and the cockatiels with a seed mixture for larger parakeets (Großsittichfutter, mifuma GmbH, Mannheim, Germany) supplemented with produce (fresh vegetables, herbs and fruits). The American kestrels were also housed in a large covered outdoor aviary with a shelter room. The Barbary falcons were tethered, with regular periods of free flight. Both falcon species were fed with mice and day-old chicks. The study was performed during early summer, with outdoor temperatures ranging from 18 °C to 22 °C during

the day and 9 °C to 13 °C during the night. Daylight length ranged from 15:35 hours to 16:25 hours.

Ethical statement

The study protocol was ethically approved by the Animal Care and Use Committee of the District Government of Saxony, Germany. All procedures were conducted in accordance with the national guidelines for research on animals. Written informed consent was obtained from the owner of the falcons prior to the study.

Clinical examination

The health status of all birds was determined with a thorough physical examination prior and during the experiment. Feces were examined by direct smear, flotation, sedimentation and cytology. Microbiologic examination of feces was done for *Salmonella* spp. on Brilliant-green phenol-red lactose sucrose-agar (BPLS) and Xylose Lactose Tergitol-agar (XLT4) (Oxoid GmbH, Wesel, Germany) after selective enrichment in Rappaport Vassiliadis broth (Oxoid GmbH, Wesel, Germany) over 24 hours ten days before the experiment started.

Iohexol administration, sample collection and plasma analysis

All birds were fasted before the study (pigeons for 4 hours; chickens for 6 hours; cockatiels for 2 hours; falcons for 6 hours), to ensure the crop was empty before iohexol was administered orally. Water was freely accessible at any time prior to and during the study. Iohexol (755 mg/ml which corresponded to 350 mg of iodine/ml; Omnipaque-350, GE Healthcare AS, Oslo, Norway) was administered at variable doses as described below via a feeding tube inserted directly into the crop to ensure complete ingestion. The feeding tube was then flushed with a five-fold amount of isotonic saline solution (0.9 % Sodium Chloride) and removed. In pigeons, blood samples were taken from the ulnar vein; in chickens, cockatiels and falcons, blood samples were taken from the jugular vein (at a maximum 0.8 % of the body mass). Blood samples were

obtained at pre-defined time intervals after the oral administration of iohexol, and stored in EDTA tubes (Sarstedt AG & Co. KG, Nümbrecht, Germany) at room temperature. About 30 minutes (min) after the last blood sample was taken, all samples were centrifuged for six min at 3250 *g* and the blood plasma was subsequently frozen at -20 °C. Storage time before analysis varied between 8 and 94 days (median 38 days). The plasma concentration of iohexol was determined by an enzyme-linked immunosorbent assay (ELISA) (FIT-GFR™ Kit (Iohexol), BioPAL Inc., Worcester, MA, USA) according to manufacturer's instructions. This assay was recently validated for iohexol determination in canine plasma samples.[24]

Determination of recommended iohexol dosage with pigeons and chickens

The recommended iohexol dosage for the assessment of intestinal permeability in birds was determined by testing three different dosages in six healthy pigeons and six healthy chickens. Therefore, two animals of each species were always involved in a 2x3 Latin square design. At each sampling period – which occurred at two-week intervals – each animal received one of three different iohexol dosages (1.0, 2.0 and 4.0 ml/kg corresponding to 755, 1.510 and 3.020 mg iohexol/kg body weight) in a fashion that every animal received every dosage once. Blood samples were taken 45, 90 and 180 min after oral iohexol administration, and plasma was analyzed for iohexol as described above.

Comparative study with cockatiels and falcons

We further analyzed six healthy cockatiels and six healthy falcons (American kestrels and Barbary falcons). Based on the results of the preliminary dose determination study (see above), 1 ml/kg was selected as the recommended dosage for the iohexol permeability test. Oral administration was performed as described above. In cockatiels and Barbary falcons, blood was collected after 45 and 90 min. Two weeks later, the trial was repeated. In American kestrels, only one blood sample could be collected after oral iohexol administration. As such, a blood sample

was collected after 45 min on the first trial day, and after 90 min on the second trial day (two weeks later).

Data analysis

All analyses were performed with commercially available statistical software (SPSS 23.0, SPSS Inc., Chicago, IL, USA; SAS® system 9.4, SAS Institute Inc., Cary, NC, USA). Descriptive statistics were generated. For pigeons and chickens, the species, doses and timepoints were compared with linear mixed effects model. Square-root transformation was used for the iohexol-concentrations to satisfy the normality assumption. The linear mixed model included fixed effects for species (pigeon, chicken), timepoint (45, 90, 180) and dose (1 ml/kg, 2 ml/kg, 4 ml/kg) together with two-way interactions of species*timepoint, species*dose and timepoint*dose. Each individual animal served as a random effect in the model. Pairwise differences between species (overall, and separately for each dose level), dose levels and time points were estimated from the model using least square means. The model fit was assessed by evaluating studentized model residuals graphically (normal QQ-plot) and by a test of normality (Kolmogorov-Smirnov test). Values of $p < 0.05$ were considered as statistically significant.

The upper 95% and 99% confidence intervals were calculated to determine the cutoff point above which the iohexol plasma concentration would be significantly higher than concentrations after an oral iohexol dosage of 1 ml/kg, similar to a previous study performed on dogs.[25] Statistical analysis and calculation of the confidence intervals in cockatiels and falcons were not performed due to the high variation among individuals.

Results

Health status

Clinical evaluation was normal for all birds at all times. Fecal examinations were negative for the presence of any endoparasites. Microbiological tests showed no evidence of *Salmonella* spp.

Dose determination study

After oral administration, iohexol plasma concentrations increased to highest levels at 180 min regardless of the species (with p-value of < 0.001 for the comparisons against both 45 min and 90 min time point). Also, at 90 min post application the iohexol plasma concentration was higher than at 45 min (p=0.001). The maximum individual concentrations occurred at either 90 or 180 min post application, both in pigeons and in chickens (**Table 1**).

When comparing the two species over the three dose levels, pigeons were shown to have higher iohexol plasma concentration levels than chickens (p=0.032). This detected difference between species is similar with each of the three dose levels (p-value for the species*dose interaction 0.461). However, the estimated difference between species, within dose level is statistically significant only at 4 ml/kg level (p=0.012). For the other two dose levels the difference between species was just above statistical significance (p=0.085 for both doses).

As said, there was no interaction between species and dose level, thus the dose levels can be compared over the two species. The 4 ml/kg dosage was shown to have higher plasma iohexol concentration levels compared to the other two dosage levels 1 ml/kg, 2 ml/kg (p-values < 0.001 and 0.022 respectively). There was no significant difference between the 1 ml/kg and 2 ml/kg dose levels (p=0.120). The highest individual iohexol plasma concentrations measured after application of 1/2/4 ml iohexol per kg were 79.79 µg/ml / 103.60 µg/ml and 203.70 µg/ml in a pigeon and 65.00 µg/ml / 132.80 µg/ml and 199.50 µg/ml in a chicken, all at 180 min. In summary, the measured iohexol plasma concentrations were higher in pigeons than in chickens and highest with the 4 ml/kg dose level compared to the other two dose levels (**Figure 1**). Adverse effects (watery droppings) were observed in two pigeons 120 min after the highest

dosage (4 ml/kg) was administered. The dosage of 1 ml/kg iohexol was selected as recommended for permeability testing in the remaining birds. In chickens, values are significantly increased after 90 min when iohexol plasma concentration is above 31.51 µg/ml (95 %) or 37.41 µg/ml (99 %). In pigeons iohexol concentrations are significantly increased after 90 min when above 53.59 µg/ml (95 %) or 61.42 µg/ml (99 %).

Comparative study

In cockatiels and falcons, iohexol plasma concentrations showed the same trends as in pigeons and chickens: plasma levels increased up to 90 min after iohexol administration in most of the tested birds. One exception was a cockatiel (no. 2), in which the iohexol plasma level dropped from 8.64 µg/ml after 45 min to 6.63 µg/ml after 90 min on the second trial. A decrease in iohexol plasma concentration was also observed in a Barbary falcon (no. 5), where the iohexol plasma level dropped from 124.10 µg/ml after 45 min to 47.95 µg/ml after 90 min on the second trial (**Table 2**).

The highest individual iohexol plasma concentration in cockatiels was 56.68 µg/ml 90 min after oral administration. In American kestrels, the highest plasma concentration measured was 102.20 µg/ml after 90 min; in Barbary falcons, it was 134.5 µg/ml after 90 min. Some of the tested birds showed wide variation in iohexol plasma concentrations from one trial day to the other (cockatiel no. 1 at 90 min; falcon no. 5 at 45 and 90 min).

Due to their small body size and problems with hematoma formation, it was only possible to obtain a single blood sample per trial day in the American kestrels – blood was sampled after 45 min on the first trial day, and after 90 min on the second trial day. In this part of the study, no iohexol-related adverse effects were seen.

Discussion

The results of the present study indicate that upon oral administration, iohexol at a dosage of 1 ml/kg is a safe and promising substance for non-invasive intestinal permeability blood tests in clinically healthy pigeons, chickens, cockatiels, American kestrels and Barbary falcons. A feeding tube was used for oral administration to ensure complete ingestion of the marker substance. This method is common in avian practice, as it is well-tolerated by the birds and does not require any sedation.[26] Drawing blood samples from birds is a relatively simple procedure, and considering that only a small amount of blood plasma or serum is needed for iohexol analysis with an ELISA, the method is suitable even for small avian species.[27] In mammals, it is common to measure recovery rates of permeability markers, typically in urine samples obtained in metabolic cages.[28] However, collecting urine samples is susceptible to fecal contamination in birds. Since birds lack a urinary bladder, the urine is held in the colon where it has contact with the feces. This can result in unnaturally high marker concentrations reported in (contaminated) urine when in fact most of the detected probe comes from the fecal matter, so that collecting pure urine samples while avoiding faecal contamination is difficult and probably not feasible in birds.[29, 30]

To determine the recommended iohexol dosage for permeability testing, we chose three different dosages according to previous studies in mammals.[13, 25, 31] The dosage of 1 ml/kg was selected as suitable for permeability testing in birds because it allowed easy measurement and interpretation of iohexol concentrations in blood without causing any observable adverse effects in the animals. Furthermore, by keeping the administered volume as low as possible, it is less likely to cause problems with regurgitation or other unexpected adverse effects such as osmotic diarrhoea. To reduce the osmolality of the hyperosmolar iohexol solution and avoid osmotic diarrhoea, we additionally administered a five-fold volume of isotonic saline solution. However, at a dosage of 4 ml/kg some of the individuals experienced diarrhoea. Compared to similar studies in healthy dogs, the results in all four avian species were consistently higher, despite using the same dosage and similar time points for blood sampling.[25] A recent study

revealed a strong correlation between results from ELISA and high-performance liquid chromatography (HPLC) methods in analysis of canine plasma.[24] Thus far, neither ELISA nor HPLC methods have been validated for iohexol detection in avian plasma. However, it is likely that a similar correlation would also exist between methods when measuring avian plasma, since iohexol is an inert and very stable molecule that is not metabolized in any species. To increase the reliability of our analyses, the ELISA was run with duplicate samples. The results of these samples were consistent between the analysis. Median plasma values varied between the different avian species after oral administration of 1 ml iohexol/kg body weight. Such differences in iohexol permeation and concentration could be a result of variation in nutrition/diet and/or the anatomy of the gastrointestinal tract of the species investigated here. For example, pigeons, belonging to the Columbiformes, only have vestigial caeca and are classified as a granivorous species. Chickens, which belong to the Galliformes, have fully-developed caeca and are classified as omnivorous. Cockatiels are representatives of the Psittaciformes, and do not have caeca; they are classified as granivorous.[32, 33] American kestrels and Barbary falcons belong to the Falconiformes, and are carnivorous with only vestigial caeca.[34]

The iohexol plasma values seem to be higher in falcons than in pigeons, chickens and cockatiels. One possible explanation could be physiological differences between carnivores (falcons) and granivores/omnivores (pigeons, chickens and cockatiels). Alternatively (or additionally), unlike the other birds in the study, the falcons were not accustomed to handling and physical restraint during oral administration and blood sampling, hence they likely experienced more stress during the procedures. Indeed, a study in rats described a positive correlation between the activity of the sympathetic nervous system (i.e. stress) and intestinal wall permeability.[35] Although American kestrels and Barbary falcons both belong to the same genus and have a similar diet and gastrointestinal anatomy, further studies are recommended to evaluate possible differences between these two falcon species.

In the presence of intestinal damage, a higher amount of the permeability marker will pass through the gut-blood-barrier, resulting in higher iohexol concentrations in the blood. Compared to values reported in this study – which focused on healthy birds – significantly increased iohexol plasma concentrations would be expected in birds affected with certain gastrointestinal diseases. Future studies are needed to confirm this assumption. In particular, birds with signs of chronic wasting syndrome should be investigated to determine how this disorder affects intestinal permeability. Birds with other common disorders such as infections with *Macrorhabdus ornithogaster* or mycobacteria, parasites of the gastrointestinal tract, proventricular dilatation disease or gastrointestinal intoxications would also provide important insights about infection and permeability.

In summary, we determined a recommended iohexol dosage for permeability testing in birds and made comparative studies in four nutritionally and anatomically different species. Due to the small number of animals analyzed in this trial, further studies are warranted to confirm the value of this iohexol blood test as a diagnostic tool for avian species in clinical practice and research.

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References

[1] Travis S, Menzies I. Intestinal permeability: functional assessment and significance. Clin Sci 1992;82:471-488.

[2] Farhadi A, Banan AL, Fields J, et al. Intestinal barrier: An interface between health and disease. J Gastroenterol Hepatol 2003;18:479-497.

[3] McKay DM, Shute A, Lopes F. Helminths and intestinal barrier function. Tissue Barriers 2017;5:e1283385-1-16.

[4] Zhang Y, Lee B, Thompson M, et al. Lactulose-mannitol intestinal permeability test in children with diarrhea caused by rotavirus and cryptosporidium. J Pediatr Gastroenterol Nutr 2000;31:16-21.

[5] Bjarnason I, MacPherson A, Hollander D. Intestinal Permeability: An Overview. Gastroenterology 1995;108:1566-1581.

[6] Clayburgh DR, Shen L, Turner JR. A porous defense: the leaky epithelial barrier in intestinal disease. Lab Invest 2004;84:282-291.

[7] Gerova VA, Stoynov SG, Katsarov DS, et al. Increased intestinal permeability in inflammatory bowel diseases assessed by iohexol test. World J Gastroenterol 2011;17:2211-2215.

[8] Bischoff SC, Barbara G, Buurman W, et al. Intestinal permeability - a new target for disease prevention and therapy. BMC Gastroenterol 2014;14:189.

[9] Forsgård RA, Korpela R, Holma R, et al. Intestinal permeability to iohexol as an in vivo marker of chemotherapy-induced gastrointestinal toxicity in Sprague-Dawley rats. Cancer Chemother Pharmacol 2016;78:863-874.

[10] Wyatt J, Vogelsang H, Hübl W, et al. Intestinal permeability and the prediction of relapse in Crohn's disease. Lancet 1993;341:1437-1439.

[11] Allenspach K, Steiner JM, Shah BN, et al. Evaluation of gastrointestinal permeability and mucosal absorptive capacity in dogs with chronic enteropathy Am J Vet Res 2006;67:479-483.

[12] Frias R, Sankari S, Westermarck E. 51 Cr-EDTA absorption blood test: an easy method for assessing small intestinal permeability in dogs J Vet Intern Med 2004;18(2):156-159.

[13] Frias R, Strube K, Ternes W, et al. Comparison of 51chromium-labeled ethylenediamine tetra-acetic acid and iohexol as blood markers for intestinal permeability testing in Beagle dogs. Vet J 2012;192:123-125.

[14] Frias R, Ouwehand A, Jaakkola UM, et al. An in vivo permeability test protocol using iohexol to reduce and refine the use of laboratory rats in intestinal damage assessment. Scand J Lab Anim Sci 2014;40:1-6.

[15] Andersen R, Stordahl A, Aase S, et al. Intestinal permeability of x-ray contrast media iodixanol and iohexol during bacterial overgrowth of small intestines in rats. Dig Dis Sci 2001;46:208-213.

[16] Ernst S, Goggins JM, David BS, et al. Comparison of iohexol and barium sulfate as gastrointestinal contrast media in mid-sized psittacine birds. J Avian Med Surg 1998;12:16-20.

[17] Williams J, Biller D, Myer W et al. Use of iohexol as a gastrointestinal contrast agent in three dogs, five cats and one bird. J Am Vet Med Assoc 1993;202:624-627.

[18] Gasthuys, E, Montesinos, A, Caekebeke, N et al. Comparative physiology of glomerular filtration rate by plasma clearance of exogenous creatinine and exo-iohexol in six different avian species. Sci Rep 2019;9;19699.

[19] Doss GA, Mans C, Johnson L et al. Diagnosis and management of inflammatory bowel disease in a harpy eagle (Harpia harpyja) with suspected fenbendazole toxicosis. J Am Vet Med Assoc 2018;252:336-342.

[20] Jones R. Wasting syndrome in captive peregrine falcons. In: Proceedings. Veterinary Medicine for Falconry into the 21st century 2014;25-26.

[21] Gilani S, Howarth GS, Kitessa SM, et al. Intestinal permeability induced by lipopolysaccharide and measured by lactulose, rhamnose and mannitol sugars in chickens. Animal 2017;11:1174-1179.

[22] Gilani S, Howarth GS, Tran CD, et al. Reduced fasting periods increase intestinal permeability in chickens. J Anim Physiol Anim Nutr 2018;102:e486-e492.

[23] Kuttappan VA, Vicuña EA, Latorre JD, et al. Evaluation of gastrointestinal leakage in multiple enteric inflammation models in chickens. Front vet sci 2015;2:66.

[24] Ortín-Piqueras V, Spillmann T, Pöytäkangas M, et al. Determination of iohexol in canine plasma - strong correlation between enzyme-linked immunosorbent assay, high-performance liquid chromatography, and neutron activation analysis. Scand J Lab Anim Sci 2018;44:1-7.

[25] Klenner S, Frias R, Coenen M, et al. Estimation of intestinal permeability in healthy dogs using the contrast medium iohexol. Vet Clin Pathol 2009;38:353-360.

[26] Coles BH. Medication and Administration of Drugs. In: Coles BH. Essentials of avian medicine and surgery. 3rd ed. Oxford, Iowa: Blackwell Pub; 2007;128-130.

[27] BioPhysics Assay Laboratory (BioPAL™), FIT-GFR™ Kit (IOHEXOL). Available at: http://www.biopal.com/pdf-downloads/manuals/iohexol-manual.pdf. Accessed June 25, 2018.

[28] Whittaker AL, Lymn KA, Howarth GS. Effects of metabolic cage housing on rat behavior and performance in the social interaction test. J Appl Anim Welf Sci 2016;19:363-374.

[29] Manangi MK, Clark FD, Coon CN. Improved colostomy technique and excrement (urine) collection device for broilers and broiler breeder hens. Poult Sci 2007;86:698-704.

[30] Laverty G, Skadhauge E. Adaptive strategies for post-renal handling of urine in birds. Comp Biochem Phys A 2008;149;246-254.

[31] Halme L, Turunen U, Tuominen J. Comparison of iohexol and lactulose-mannitol tests as markers of disease activity in patients with inflammatory bowel disease. Scand J Clin Lab Invest 2009;60:695-701.

[32] Lumeij, JT. Gastroenterology. In Ritchie BW, Harrison GJ, Harrison LR. Essentials of avian medicine and surgery. 3rd ed. Oxford, Iowa: Blackwell Pub, 2007;506.

[33] Jones D. Feeding ecology of the cockatiel, *Nymphicus hollandicus*, in a grain-growing area. Wildlife Res 1987;14:105-115.

[34] Ford S. Raptor gastroenterology. J Exot Pet Med 2010;19:140-150.

[35] Santisteban MM, Qi Y, Zubcevic J, et al. Hypertension-linked pathophysiological alterations in the gut. Circ Res 2017;120:312-323.

Tables

Table 1: Iohexol concentrations in plasma of pigeons and chickens after oral administration of three different dosages of Omnipaque-350.

	Iohexol (µg/ml)								
Dosage	**1 ml/kg**			**2 ml/kg**			**4 ml/kg**		
Time	**45 min**	**90 min**	**180 min**	**45 min**	**90 min**	**180 min**	**45 min**	**90 min**	**180 min**
Pigeon 1	16.24	28.81	20.16	23.66	32.80	103.60	31.50	79.59	175.20
Pigeon 2	36.97	47.43	59.80	36.59	44.36	53.25	54.66	81.97	203.70

Pigeon 3	32.13	52.95	79.79	39.44	36.50	67.83	37.27	48.84	123.60
Pigeon 4	23.41	33.93	59.98	34.11	52.77	70.36	31.48	50.86	134.80
Pigeon 5	44.51	53.21	34.31	34.46	56.93	66.30	23.71	30.54	52.72
Pigeon 6	13.45	22.66	50.12	21.91	28.38	31.99	16.42	27.00	55.82
Median	**27.77**	**40.68**	**54.96**	**34.29**	**40.43**	**67.07**	**31.49**	**49.85**	**129.2**
Chicken 1	18.98	13.43	65.00	18.96	16.66	132.80	14.92	29.38	31.25
Chicken 2	28.17	35.38	51.53	26.26	22.00	37.68	21.49	36.32	199.50
Chicken 3	4.52	7.96	5.28	11.39	13.03	11.65	2.47	6.18	8.55
Chicken 4	15.03	26.78	47.78	31.04	44.80	49.93	14.47	47.87	63.60
Chicken 5	8.14	18.32	21.80	14.47	37.44	28.48	14.80	22.57	24.01
Chicken 6	10.91	24.86	45.04	12.19	33.42	17.37	27.72	60.88	105.50
Median	**12.97**	**21.59**	**46.41**	**16.72**	**27.71**	**33.08**	**14.86**	**32.85**	**47.42**

Table 2: Iohexol concentrations in plasma of cockatiels and falcons (Falcons 1-4: American kestrels; falcons 5-6: Barbary falcons) after oral administration of 1 ml/kg Omnipaque-350. ND = not determined

	Iohexol (µg/ml)

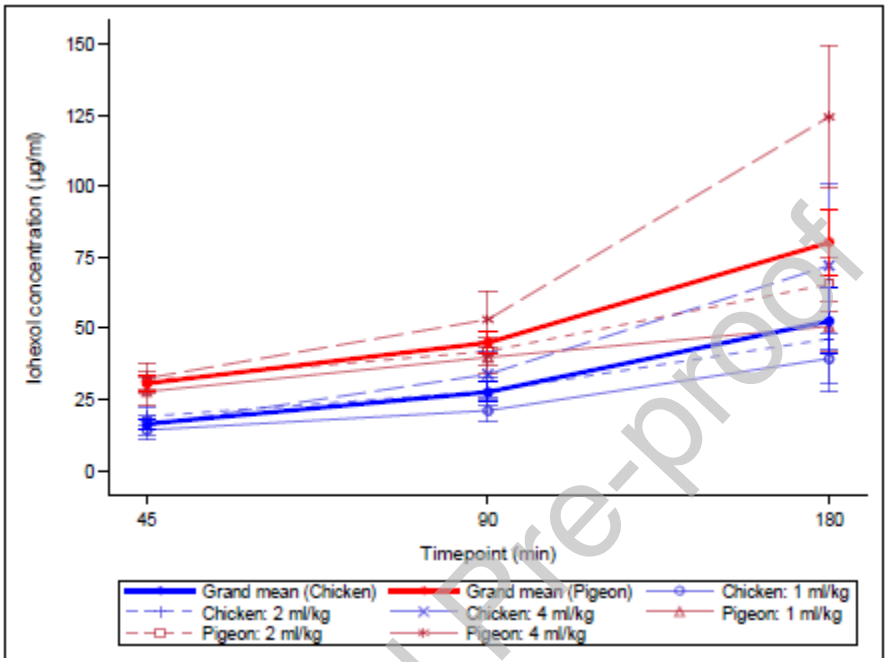
Time	**45 min**		**90 min**	
Trial	**Day 1**	**Day 2**	**Day 1**	**Day 2**
Cockatiel 1	12.46	10.21	56.68	13.91
Cockatiel 2	11.47	8.64	18.75	6.63
Cockatiel 3	22.91	26.90	31.75	43.94
Cockatiel 4	16.79	23.37	34.93	44.70
Cockatiel 5	10.90	14.24	32.30	23.34
Cockatiel 6	14.24	21.84	25.89	38.35
Median	**14.24**		**32.03**	
Falcon 1	43.33	ND	ND	19.30
Falcon 2	99.37	ND	ND	102.20
Falcon 3	25.06	ND	ND	44.62
Falcon 4	22.92	ND	ND	33.02
Falcon 5	62.17	124.10	134.50	47.95
Falcon 6	52.28	31.37	63.96	113.80
Median	**47.81**		**55.96**	

Figure Captions

Declaration of interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Figure 1: Iohexol plasma concentrations in pigeons and chickens using three different dosages of Omnipaque-350.



CRedit Author Statement

Franziska R. Wilhelm: Writing – Original draft preparation, Conceptualization, Investigation, Methodology, Data curation
Maria-E. Krautwald-Junghanns: Resources
Victoria Ortín Piqueras: Advice in laboratory analysis
Jouni Junilla: Statistical advice
Kerstin Cramer: Statistical advice
Richard Forsgard: Revision
Rafael Frias: Revision
Thomas Spillmann: Writing – Review and Editing, Resources, Supervision
Volker Schmidt: Writing – Review and Editing, Conceptualization, Supervision